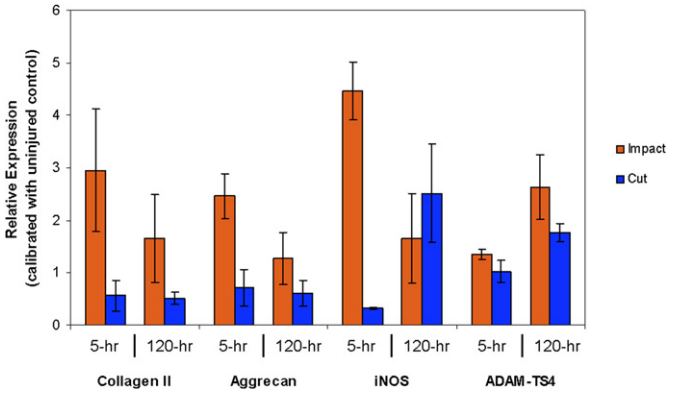
Conclusions: In this work, we showed for the first time that multiscale large field imaging (Macroscopy) combined to multimodality approaches (SHG-TCSPC) could be an innovative and non invasive technique to monitor the state of network collagen in biomedical studies such as cartilage tissue engineering.

247

A MODEL FOR TESTING THE EFFECTS OF INJURY AND REPAIR USING ENGINEERED CARTILAGE TISSUE ANALOGS

B. Mohanraj, W.C. Strutz, R.L. Mauck, G.R. Dodge. *Univ. of Pennsylvania, Philadelphia, PA, USA*

Purpose: Articular cartilage consists of unique extracellular matrix (ECM) which function to distribute loads in synovial joints. When injured, it cannot repair effectively and is increasingly thought that injury or trauma, even the smallest may initiate degenerative changes (i.e. OA). The goal was to devise a platform to test chondrocyte's response to injury using a cartilage tissue analogue (CTA) after a rapid blunt force trauma and/or laceration. The aim was to measure biosynthetic and catabolic responses in chondrocytes, in a reproducible in vitro model, following injury. The long term goal was to provide a platform to study chondrocyte function and to test therapeutics.
Methods: CTA Preparation- Chondrocytes were isolated from juvenile bovine (1-3 months old) knees and CTA generated. Briefly, cartilage was dissected and digested overnight with collagenase. The CTA model is based on using plates coated with hydrogel, poly (2-hydroxyethyl methacrylate), which prevents cell adherence. The cells coalesce and form a single mass which grows quickly and maintains a cartilage phenotype for long periods in culture. In these experiments chondrocytes were seeded at 1-2x10^6 cells/ well in 96 well plate and cultured in DMEM with 10% FBS for at least 6 -10 weeks to allow for substantial ECM production. Sample CTA Groups- (1) Uninjured control, (2) Uninjured treated with 10 ng/mL IL-1a (positive control), (3) Cut CTAs, and (4) Impacted CTAs, were harvested 5 hours and 5 days post-injury. Injury- an Instron 5848 was used to determine the CTA height while resting on a flat surface. The impact injury regimen was 75% strain over 1 sec., held for 3 sec., then released. 3 full thickness lacerations were made through the CTA ('Cut'). After impact or laceration, CTAs were transferred to medium for additional culture periods or immediately flash frozen for gene expression analysis. At corresponding harvest times CTA were rushed into a powder, and RNA was purified followed by qPCR using the Bio-Rad CFX384 and iQ Sybr GreenSupermix. Data was analyzed using $2^{-\Delta\Delta C}{}_T$ method to obtain relative expression with data presented as percent of untreated or un-injured controls.
Results: The effects of impact injury on ECM gene expression over an extended period of three time points 0, 24, and 120 h was evaluated.Collagen and aggrecan were down-regulated at 24 h following impact and IL-1 treatment. However, at 120 h, collagen was markedly up-regulated after injury, as was aggrecan but to a lesser extent. IL-1a treatment continued to down-regulate aggrecan at 120h, while collagen showed a slight increase (24%). Perlecan was up-regulated in response to injury at 120h (~30%) and to IL-1a at both time points. Comparisons between cut and impacted CTAs at 5h showed an upregulation of collagen and aggrecan expression ~3-fold and ~2.5-fold, respectively. (Fig 1).



However, this upregulation was diminished at 120h. In contrast, in cut CTAs, aggrecan and collagen II was only slightly up-regulated and remained at constant expression levels at 5 and 120h. The stress related gene iNOS was markedly up-regulated at 5h in impacted CTAs (~ 4.5X), and this increase was reduced nearly to uninjured at 120 h. In contrast, cut CTAs had low level expression of iNOS at 5h, but was up-regulated 2.5-fold at 120h. ADAM-TS4 expression was similarly unaffected at 5 h for both cut and impacted CTAs but was up-regulated in impacted samples (~2.6X) at 120h.
Conclusion: A single, rapid, uniaxial compressive load on an engineered cartilage construct was used to generate a model of injury and demonstrate the changes on chondrocyte gene expression. While proteases tested and stress indicators are involved in the cellular injury response after both injury types, chondrocytes appear to respond to compressive injury by increasing the biosynthesis of certain ECM genes at longer time points tested. Extending the recovery periods will reveal to what degree these mechanically-induced injuries heal or become degenerative. This model establishes a platform to test engineered cartilage surrogate's response to injury and ultimately identify pathways involved in repair and test therapeutics.

248

CARTILAGE REPAIR FOR STEROID-INDUCED OSTEONECROSIS OF THE KNEE JOINT

H. Tsukiyama, M. Kobayasi, R. Arai, H. Harada, Y. Nakagawa. *Kyoto Univ. Hosp., Kyoto, Japan*

Purpose: Surgical treatment for steroid-induced osteonecrosis is challenging one. Purpose of this study was to evaluate the outcome of osteochondral autogenous transfer (OAT) to steroid-induced osteonecrosis of the knee joint.
Methods: Thirteen knees (10 patients) of streroid-induced osteonecrosis of femoral condyles were consecutively treated with OAT between year of 2004 and 2011. Average age at the surgery was 31.6 years old. The primary disease was SLE in 6 patients, chronic active EB virus infection in 1 patient, chronic glomeralonephtitis in 1 patient, MCTD in 1patient, and intestinal Bechet`s disease in 1 patient. The mean highest corticosteroid dosage per day was 523 mg/day (range; , 30 to 1000mg/day). The average follow up period was 41 month (range; , 4 to 75 months). JOA scoring system was used to evaluate clinical result.
Results: The average JOA score improved from 67.5 points preoperatively (range; , 45 to 80) to 91.4 points (range; , 85 to 100) postoperatively. Almost all patients could do Japanese style sitting post operatively. We replaced damaged cartilage together with necrotized bone on the weight-bearing area with osteochondral autograft as much as possible. We also removed as much necrotized bone as possible, and grafted iliac bone if the area of necrotized bone was larger than that where osteochondral plugs could cover. Iliac bone grafting was necessary in 2 knees. Osteotomy was not performed. Necrosis of the femoral neck was seen in 6 patients. There was no major complication such as infection.
Conclusions: The present study shows that OAT to steroid-induced osteonecrosis of the knee joint was one surgical option resulting in good short-term outcome.

249

CARTILAGE REPAIR OF THE ANKLE WITH MICROFRACTURING OR AUTOLOGOUS CHONDROCYTE IMPLANTATION/MATRIX ASSOCIATED AUTOLOGOUS CHONDROCYTE IMPLANTATION: FOLLOW UP FROM 1 TO 14 YEARS

M. Brix [1], C. Chiari [1], S. Nehrer [2], R. Windhager [1], S. Domayer [1]. [1] *Med. Univ. of Vienna, Vienna, Austria;* [2] *Danube Univ. Krems, Krems, Austria*

Purpose: Ideal treatment of cartilage defects of the ankle is still controversial. As opposed to the knee, where the autologous chondrocyte implantation (ACI) or matrix associated autologous chondrocyte implantation (MACI) has become an established treatment for cartilage defects, little is known about ACI/MACI in the ankle.
The aim of this study was the clinical follow up of a study group treated with ACI/MACI and one study group treated with Microfracture (MFX).
Methods: Altogether, 44 patients (24 female; 20 male) with articular cartilage lesions of the ankle consented to this prospective case series. 19